

## Claims

1. A method of preparing amplified RNA sequences present in one or more than one target polynucleotide that is single stranded or made single stranded, comprising
  - a) forming double stranded cDNA templates containing sequences present in said target polynucleotide, wherein said sequences are operably linked to a promoter, by
    - i) annealing said single stranded target polynucleotide with a plurality of first oligonucleotides, each comprising a random primer sequence, to form a first complex,
    - ii) synthesizing a first strand cDNA by reverse transcription of said first complex and adding a homopolymer tail to said first strand cDNA by use of terminal deoxyribonucleotidyl transferase activity,
    - iii) optionally degrading first oligonucleotides not used in i) or ii) above with exonuclease activity,
    - iv) annealing said first strand cDNA, after denaturing the mRNA/cDNA hybrid or degrading the RNA from said hybrid, with a second oligonucleotide comprising a primer sequence, complementary to said homopolymer tail and operably linked to a promoter region, to form a population of second complexes, and
    - v) forming double stranded cDNA templates from said population of second complexes with DNA polymerase activity; and
  - b) transcribing said cDNA templates with an RNA polymerase capable of initiating transcription via said promoter region to produce amplified RNA (aRNA) containing sequences of said target polynucleotide.
2. The method of claim 1 wherein said target polynucleotide is in an RNA preparation containing mRNA, tRNA, and rRNA.
3. The method of claim 2 wherein said RNA preparation is an FFPE derived sample.

4. The method of claim 3 wherein said RNA preparation is enriched for polyadenylated mRNA molecules.

5. The method of claim 1 wherein said target polynucleotide is RNA from a breast cancer cell sample.

5         6. The method of claim 1 wherein said random primer sequence comprises at least about six random nucleotides.

7. The method of claim 6 wherein said random primer sequence comprises at least about nine random nucleotides.

10       8. The method of claim 1 wherein said DNA polymerase activity is DNA dependent.

9. The method of claim 8 wherein said DNA dependent polymerase activity is selected from exonuclease deficient Klenow, Taq polymerase activities, and combinations thereof.

15       10. The method of claim 1 wherein the production of amplified RNA sequences present in one or more than one target polynucleotide is increased by preparing additional double stranded DNA templates, comprising all or part of the sequence of the aRNA, and initiating transcription from the additional templates, said method comprising

annealing said aRNA to a third oligonucleotide comprising a primer region to form a third complex,

20       synthesizing the first strand of said additional double stranded DNA templates by reverse transcription of said third complex,

annealing said first strand of additional DNA templates, after denaturing the aRNA/DNA hybrids or degrading the aRNA from said hybrids, with said second oligonucleotide comprising an operably linked promoter region to form a fourth complex,

25       forming additional double stranded DNA templates from said fourth complex with DNA dependent DNA polymerase activity, and

transcribing said double stranded DNA templates with an RNA polymerase capable of initiating transcription via said promoter region to produce additional amplified RNA (aRNA) containing sequences of said target polynucleotide,

5 wherein the above annealing, synthesizing, annealing, forming and/or transcribing components of the method are optionally repeated to further amplify said RNA sequences complementary to one or more than one target polynucleotide.

11. The method of claim 10 wherein said third oligonucleotide comprises a random primer region.

10 12. The method of claim 11 wherein said random primer region comprises at least about six random nucleotides.

13. The method of claim 12 wherein said random primer region comprises at least about nine random nucleotides.

14. The method of claim 10 wherein said DNA dependent DNA polymerase activity comprises exonuclease deficient Klenow and Taq polymerase activities.

15 15. The method of claim 10 wherein said third oligonucleotide comprises a known primer sequence.

16. The method of claim 15 wherein said known primer sequence is complementary to the 3' region of said aRNA.

20 17. A method of amplifying RNA sequences complementary to, or present in, one or more than one target polynucleotide that is single stranded or made single stranded, comprising

a) forming double stranded cDNA templates containing sequences present in said target polynucleotide, wherein said sequences are operably linked to a promoter region, by

25 i) annealing said single stranded target polynucleotide with a plurality of first oligonucleotides, each comprising a random primer sequence, to form a first complex,

ii) synthesizing a first strand cDNA by reverse transcription of said first complex and adding a homopolymer tail to said first strand cDNA by use of terminal deoxyribonucleotidyl transferase activity,

5       iii) optionally degrading first oligonucleotides not used in i) or ii) above with exonuclease activity,

iv) annealing said first strand cDNA, after denaturing the mRNA/cDNA hybrid or degrading the RNA from said hybrid, with a second oligonucleotide comprising a primer sequence, complementary to said homopolymer tail and operably linked to a promoter region, to form a population of second complexes, and

10       v) forming double stranded cDNA templates from said population of second complexes with DNA dependent DNA polymerase activity; and

b) transcribing said cDNA templates with an RNA polymerase capable of initiating transcription via said promoter region to produce amplified RNA (aRNA) containing sequences complementary to said target polynucleotide;

15       c) forming additional double stranded DNA templates from said aRNA by

i) annealing said aRNA with a third oligonucleotide comprising a primer sequence operably linked to a promoter region to form a third complex,

ii) synthesizing the first strand of said additional DNA template by reverse transcription of said third complex,

20       iii) annealing said first strand of additional DNA template, after denaturing the aRNA/DNA hybrid or degrading the aRNA from said hybrid, with said second oligonucleotide to form a population of fourth complexes, and

iv) forming additional double stranded DNA templates from said population of fourth complexes with DNA dependent DNA polymerase activity; and

25       d) transcribing said additional DNA templates with an RNA polymerase capable of initiating transcription via the promoter region of said third oligonucleotide to produce amplified RNA (aRNA) containing sequences complementary to said target

polynucleotide or via the promoter region of said second oligonucleotide to produce aRNA containing sequences of said target polynucleotide.

18. The method of claim 17 wherein said formation of additional double stranded DNA templates from said aRNA further comprises degrading third oligonucleotides not  
5 used in c) i) or c) ii) with exonuclease activity before forming additional double stranded DNA templates.

19. The method of claim 17 wherein said target polynucleotide is in an RNA preparation containing mRNA, tRNA, and rRNA.

20. The method of claim 19 wherein said RNA preparation is an FFPE derived  
10 sample.

21. The method of claim 20 wherein said RNA preparation is enriched for polyadenylated mRNA molecules.

22. The method of claim 17 wherein said target polynucleotide is RNA from a breast cancer cell sample.

23. The method of claim 17 wherein said random primer sequence comprises at  
15 least about six random nucleotides.

24. The method of claim 23 wherein said random primer sequence comprises at least about nine random nucleotides.

25. The method of claim 17 wherein said DNA dependent DNA polymerase  
20 activity comprises exonuclease deficient Klenow and Taq polymerase activities.

26. The method of claim 17 wherein said third oligonucleotide comprises a random primer sequence.

27. The method of claim 26 wherein said random primer sequence comprises at least about six random nucleotides.

28. The method of claim 27 wherein said random primer sequence comprises at  
25 least about nine random nucleotides.

29. The method of claim 17 wherein said third oligonucleotide comprises a known primer sequence.

30. The method of claim 29 wherein said known primer sequence is complementary to the 3' region of said aRNA.

5           31. The method of claim 1 wherein said first oligonucleotide comprises a T7 promoter region.

32. The method of claim 17 wherein said third oligonucleotide comprises a T3 or SP6 promoter region.

10           33. The method of claim 17 wherein said first oligonucleotide comprises a T7 promoter region.